

SEQUENCE PROTOCOL

<110> Degussa-Hüls AG

5 <120> Process for the fermentative preparation of
L-amino acids using strains of the
Enterobacteriaceae family.

<130> 000613 BT

10

<140>

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<160> 4

15

<170> PatentIn Ver. 2.1

<210> 1

<211> 1719

20

<212> DNA

<213> *Escherichia coli*

<220>

<221> CDS

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<222> (1)..(1716)

<223> *poxB*

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ggg gtg aaa cgc atc tgg gga gtc aca ggc gac tct ctg aac ggt ctt 96

Gly Val Lys Arg Ile Trp Gly Val Thr Gly Asp Ser Leu Asn Gly Leu

35 20 25 30

agt gac agt ctt aat cgc atg ggc acc atc gag tgg atg tcc acc cgc 144
Ser Asp Ser Leu Asn Arg Met Gly Thr Ile Glu Trp Met Ser Thr Arg
35 40 45

40 cac gaa gaa gtg gcg gcc ttt gcc gct ggc gct gaa gca caa ctt agc 192
His Glu Glu Val Ala Ala Phe Ala Ala Gly Ala Glu Ala Gln Leu Ser
50 55 60

gga gaa ctg gcg gtc tgc gcc gga tcg tgc ggc ccc ggc aac	ctg cac	240
Gly Glu Leu Ala Val Cys Ala Gly Ser Cys Gly Pro Gly Asn	Leu His	
65	70	75
5 tta atc aac ggc ctg ttc gat tgc cac cgc aat cac gtt ccg	gta ctg	288
Leu Ile Asn Gly Leu Phe Asp Cys His Arg Asn His Val Pro	Val Leu	
85	90	95
gcg att gcc gct cat att ccc tcc agc gaa att ggc agc	ggc tat ttc	336
10 Ala Ile Ala Ala His Ile Pro Ser Ser Glu Ile Gly Ser	Gly Tyr Phe	
100	105	110
cag gaa acc cac cca caa gag cta ttc cgc gaa tgt agt	cac tat tgc	384
Gln Glu Thr His Pro Gln Glu Leu Phe Arg Glu Cys Ser	His Tyr Cys	
15 115	120	125
gag ctg gtt tcc agc ccg gag cag atc cca caa gta ctg	gcg att gcc	432
Glu Leu Val Ser Ser Pro Glu Gln Ile Pro Gln Val Leu Ala	Ile Ala	
130	135	140
20 atg cgc aaa gcg gtg ctt aac cgt ggc gtt tcg gtt gtc	gtg tta cca	480
Met Arg Lys Ala Val Leu Asn Arg Gly Val Ser Val Val	Leu Pro	
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25 ggc gac gtg gcg tta aaa cct gcg cca gaa ggg gca acc	atg cac tgg	528
Gly Asp Val Ala Leu Lys Pro Ala Pro Glu Gly Ala Thr Met	His Trp	
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30 tat cat gcg cca caa cca gtc gtg acg ccg gaa gaa gaa	gag tta cgc	576
Tyr His Ala Pro Gln Pro Val Val Thr Pro Glu Glu Glu	Leu Arg	
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35 aaa ctg gcg caa ctg ctg cgt tat tcc agc aat atc	gcc ctg atg tgt	624
Lys Leu Ala Gln Leu Leu Arg Tyr Ser Ser Asn Ile Ala	Leu Met Cys	
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40 ggc agc ggc tgc gcg ggg gcg cat aaa gag tta gtt gag	ttt gcc ggg	672
Gly Ser Gly Cys Ala Gly Ala His Lys Glu Leu Val Glu	Phe Ala Gly	
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Lys Ile Lys Ala Pro Ile Val His Ala Leu Arg Gly Lys	Glu His Val	
225	230	235
240		

	gaa tac gat aat ccg tat gat gtt gga atg acc ggg tta atc ggc ttc	768		
	Glu Tyr Asp Asn Pro Tyr Asp Val Gly Met Thr Gly Leu Ile Gly Phe			
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5	tcg tca ggt ttc cat acc atg atg aac gcc gac acg tta gtg cta ctc	816		
	Ser Ser Gly Phe His Thr Met Met Asn Ala Asp Thr Leu Val Leu Leu			
	260	265	270	
10	ggc acg caa ttt ccc tac cgc gcc ttc tac ccg acc gat gcc aaa atc	864		
	Gly Thr Gln Phe Pro Tyr Arg Ala Phe Tyr Pro Thr Asp Ala Lys Ile			
	275	280	285	
15	att cag att gat atc aac cca gcc agc atc ggc gct cac agc aag gtg	912		
	Ile Gln Ile Asp Ile Asn Pro Ala Ser Ile Gly Ala His Ser Lys Val			
	290	295	300	
20	gat atg gca ctg gtc ggc gat atc aag tcg act ctg cgt gca ttg ctt	960		
	Asp Met Ala Leu Val Gly Asp Ile Lys Ser Thr Leu Arg Ala Leu Leu			
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25	cca ttg gtg gaa gaa aaa gcc gat cgc aag ttt ctg gat aaa gcg ctg	1008		
	Pro Leu Val Glu Glu Lys Ala Asp Arg Lys Phe Leu Asp Lys Ala Leu			
	325	330	335	
30	gaa gat tac cgc gac gcc cgc aaa ggg ctg gac gat tta gct aaa ccg	1056		
	Glu Asp Tyr Arg Asp Ala Arg Lys Gly Leu Asp Asp Leu Ala Lys Pro			
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35	agc gag aaa gcc att cac ccg caa tat ctg gcg cag caa att agt cat	1104		
	Ser Glu Lys Ala Ile His Pro Gln Tyr Leu Ala Gln Gln Ile Ser His			
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40	ttt gcc gcc gat gac gct att ttc acc tgt gac gtt ggt acg cca acg	1152		
	Phe Ala Ala Asp Asp Ala Ile Phe Thr Cys Asp Val Gly Thr Pro Thr			
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45	gtg tgg gcg gca cgt tat cta aaa atg aac ggc aag cgt cgc ctg tta	1200		
	Val Trp Ala Ala Arg Tyr Leu Lys Met Asn Gly Lys Arg Arg Leu Leu			
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50	ggt tcg ttt aac cac ggt tcg atg gct aac gcc atg ccg cag gcg ctg	1248		
	Gly Ser Phe Asn His Gly Ser Met Ala Asn Ala Met Pro Gln Ala Leu			
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10	atg	aaa	ctg	cca	gtg	aaa	att	gtc	gtc	ttt	aac	aac	agc	gtg	ctg	ggc	1392
	Met	Lys	Leu	Pro	Val	Lys	Ile	Val	Val	Phe	Asn	Asn	Ser	Val	Leu	Gly	
	450			455				460									
15	ttt	gtg	gct	atg	gag	atg	aaa	gct	ggt	ggc	tat	ttg	act	gac	ggc	acc	1440
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20	gaa	cta	cac	gac	aca	aac	ttt	gcc	cgc	att	gcc	gaa	gct	tgc	ggc	att	1488
	Glu	Leu	His	Asp	Thr	Asn	Phe	Ala	Arg	Ile	Ala	Glu	Ala	Cys	Gly	Ile	
	485			490				495									
25	acg	ggt	atc	cgt	gta	gaa	aaa	gct	tct	gaa	gtt	gat	gaa	gcc	ctg	caa	1536
	Thr	Gly	Ile	Arg	Val	Glu	Lys	Ala	Ser	Glu	Val	Asp	Glu	Ala	Leu	Gln	
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30	cgc	gcc	tcc	tcc	atc	gac	ggt	ccg	gtg	ttg	gtg	gat	gtg	gtg	gtc	gcc	1584
	Arg	Ala	Phe	Ser	Ile	Asp	Gly	Pro	Val	Leu	Val	Asp	Val	Val	Val	Ala	
	515			520				525									
35	aaa	gaa	gag	tta	gcc	att	cca	ccg	cag	atc	aaa	ctc	gaa	cag	gcc	aaa	1632
	Lys	Glu	Glu	Leu	Ala	Ile	Pro	Pro	Gln	Ile	Lys	Leu	Glu	Gln	Ala	Lys	
	530			535				540									
40	ggt	ttc	agc	ctg	tat	atg	ctg	cgc	gca	atc	atc	agc	gga	cgc	ggt	gat	1680
	Gly	Phe	Ser	Leu	Tyr	Met	Leu	Arg	Ala	Ile	Ile	Ser	Gly	Arg	Gly	Asp	
	545			550				555							560		
45	gaa	gtg	atc	gaa	ctg	gct	aaa	aca	aac	tgg	cta	agg	taa				1719
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<212> PRT

<213> Escherichia coli

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10 Ser Asp Ser Leu Asn Arg Met Gly Thr Ile Glu Trp Met Ser Thr Arg

35 40 45

His Glu Glu Val Ala Ala Phe Ala Ala Gly Ala Glu Ala Gln Leu Ser

50 55 60

15

Gly Glu Leu Ala Val Cys Ala Gly Ser Cys Gly Pro Gly Asn Leu His

65 70 75 80

Leu Ile Asn Gly Leu Phe Asp Cys His Arg Asn His Val Pro Val Leu

20 85 90 95

Ala Ile Ala Ala His Ile Pro Ser Ser Glu Ile Gly Ser Gly Tyr Phe

100 105 110

25

Gln Glu Thr His Pro Gln Glu Leu Phe Arg Glu Cys Ser His Tyr Cys

115 120 125

30

Glu Leu Val Ser Ser Pro Glu Gln Ile Pro Gln Val Leu Ala Ile Ala

130 135 140

Met Arg Lys Ala Val Leu Asn Arg Gly Val Ser Val Val Val Leu Pro

145 150 155 160

35

Gly Asp Val Ala Leu Lys Pro Ala Pro Glu Gly Ala Thr Met His Trp

165 170 175

Tyr His Ala Pro Gln Pro Val Val Thr Pro Glu Glu Glu Leu Arg

180 185 190

40 Lys Leu Ala Gln Leu Leu Arg Tyr Ser Ser Asn Ile Ala Leu Met Cys

195 200 205

Gly Ser Gly Cys Ala Gly Ala His Lys Glu Leu Val Glu Phe Ala Gly

210 215 220

Lys Ile Lys Ala Pro Ile Val His Ala Leu Arg Gly Lys Glu His Val
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5 Glu Tyr Asp Asn Pro Tyr Asp Val Gly Met Thr Gly Leu Ile Gly Phe
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Ser Ser Gly Phe His Thr Met Met Asn Ala Asp Thr Leu Val Leu Leu
260 265 270

10 Gly Thr Gln Phe Pro Tyr Arg Ala Phe Tyr Pro Thr Asp Ala Lys Ile
275 280 285

Ile Gln Ile Asp Ile Asn Pro Ala Ser Ile Gly Ala His Ser Lys Val
15 290 295 300

Asp Met Ala Leu Val Gly Asp Ile Lys Ser Thr Leu Arg Ala Leu Leu
305 310 315 320

20 Pro Leu Val Glu Glu Lys Ala Asp Arg Lys Phe Leu Asp Lys Ala Leu
325 330 335

Glu Asp Tyr Arg Asp Ala Arg Lys Gly Leu Asp Asp Leu Ala Lys Pro
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25 Ser Glu Lys Ala Ile His Pro Gln Tyr Leu Ala Gln Gln Ile Ser His
355 360 365

Phe Ala Ala Asp Asp Ala Ile Phe Thr Cys Asp Val Gly Thr Pro Thr
30 370 375 380

Val Trp Ala Ala Arg Tyr Leu Lys Met Asn Gly Lys Arg Arg Leu Leu
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35 Gly Ser Phe Asn His Gly Ser Met Ala Asn Ala Met Pro Gln Ala Leu
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Gly Ala Gln Ala Thr Glu Pro Glu Arg Gln Val Val Ala Met Cys Gly
420 425 430

40 Asp Gly Gly Phe Ser Met Leu Met Gly Asp Phe Leu Ser Val Val Gln
435 440 445

Met Lys Leu Pro Val Lys Ile Val Val Phe Asn Asn Ser Val Leu Gly

450 455 460

Phe Val Ala Met Glu Met Lys Ala Gly Gly Tyr Leu Thr Asp Gly Thr
5 465 470 475 480

Glu Leu His Asp Thr Asn Phe Ala Arg Ile Ala Glu Ala Cys Gly Ile
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10 Thr Gly Ile Arg Val Glu Lys Ala Ser Glu Val Asp Glu Ala Leu Gln
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Arg Ala Phe Ser Ile Asp Gly Pro Val Leu Val Asp Val Val Ala
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15 Lys Glu Glu Leu Ala Ile Pro Pro Gln Ile Lys Leu Glu Gln Ala Lys
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<210> 3

<211> 1454

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35 <223> Mutagenic DNA

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<222> (1)..(56)

40 <223> Technical DNA/residues of the polylinker sequence

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<222> (57)..(577)

<223> Part of the 5' region (poxB1) of the poxB gene

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5 <222> (578)..(646)

<223> Technical DNA/residues of the polylinker sequence

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10 <222> (647)..(1398)

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15 <222> (1399)..(1454)

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gcccggatc gtgcggcccc ggcaacctgc acttaatcaa cggcctgttc gattgccacc 240

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ggcgatttc ctctcagtag tgcagatgaa actgccagtg aaaattgtcg tcttaacaa 1080

cagcgtgctg ggcttgggtt cgatggagat gaaagctggt ggctatttgc ctgacggcac 1140

cgaactacac gacacaaact ttgccccat tgccgaagcg tgccgcatta cgggtatccg 1200

tgtagaaaaa gcgtctgaag ttgatgaagc cctgcaacgc gccttctcca tcgacggtcc 1260

40 ggtgttgggt gatgtgggttgg tcgccaaaga agagttagcc attccaccgc agatcaaact 1320

cgaacaggcc aaaggttca gcctgtat gctgcgcgc atcatcagcg gacgcgggtga 1380

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1454

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5 <213> *Escherichia coli*

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10 <223> Start codon of the delta poxB allele

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<222> (1)..(605)

15 <223> 5' region of the delta poxB allele

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<222> (606)..(674)

20 <223> Technical DNA/residues of the polylinker sequence

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<222> (675)..(1445)

25 <223> 3' region of the delta poxB allele

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<222> (1446)..(1448)

30 <223> Stop codon of the delta poxB allele

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35 accatcgagt ggatgtccac ccgccacgaa gaagtggcgg ccttgcgc tggcgctgaa 180
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taaggtaa 1448

15